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Monofunctionalization of Calix[4]arene Tetracarboxylic Acid at the Upper Rim with Isothiocyanate Group: First Bifunctional Chelating Agent for Alpha-Emitter Ac-225

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Abstract

A procedure is reported for synthesizing a novel, water-soluble bifunctional chelating agent derived from calix[4]arene. This chelate features tetracarboxylic acid groups at the lower rim as an actinium-225 ionophore, and an isothiocyanate functional group at the upper rim for labeling of the N-terminus of monoclonal antibodies through thiourea linkage.

Keywords

actinium-225; bifunctional chelating agents; calixarenes; isothiocyanate

For radioimmunotherapy of cancer, it is well established that alpha (α) particles from radionuclide labels are more effective cytotoxic agents than beta (β) particles. This is because α particles dissipate a larger amount of energy over a shorter range (few cell diameters), and thus are more effective in cell killing than are β particles.^{1,2} Among the α -emitting radionuclides considered suitable for radioimmunotherapy of cancer is actinium-225 ($t_{1/2} = 10$ d), which decays through a rapid chain of daughter products to a stable product and emits four α particles, two β particles, and several gamma-ray (γ) photons with a combined energy of about 28 MeV.³ Applications in radioimmunotherapy require Ac-225 to be bound to a protein that specifically targets cancer cells. This application also requires a bifunctional complexing agent that tightly binds Ac-225 on one side and can be linked to protein on the other side. This bifunctional complexing agent must be stable under physiological conditions. We have recently reported⁴ that *t*-butylcalix[4]arene tetracarboxylic acid and *t*-butylcalix[6]arene hexacarboxylic acid showed high efficiency for binding actinium-225 under neutral and weak acid conditions. The calix[4]arene platform exhibited better selectivity and higher kinetic stability than calix[6]arene analog with respect to acid and serum abundant cations (such as Na⁺, K⁺, Mg²⁺, Ca²⁺, and Zn²⁺) promoted pathways. Calix[4]arene tetracarboxylic acid appeared to be a suitable candidate as an actinium-225 complexing agent for applications in immunotherapy. Medicinal applications require a water soluble immunoconjugate. Modeling studies reported recently by Reinhoudt group⁵ revealed that in the Ac³⁺ complex of calix[4]arene tetracarboxylic acid, the cation was bound inside the cavity with average coordination distances of Ac---O_{phenol} (2.78 Å) and Ac---O₂C (2.47 Å), the carboxylate groups behave as monodentate. We report herein the first design of a water-soluble bifunctional chelating agent (Compound **6** in Scheme 1) for binding actinium-225 for labeling of monoclonal antibodies. The four carboxylic acid

groups, fixed in conic conformation at the lower rim of the calix[4]arene platform, act as an actinium ionophore. The monofunctionalized isothiocyanato group of the upper rim is designed to facilitate the combination of this C-functionalized bifunctional chelator with N-terminus of desired monoclonal antibody *via* thiourea linkage.

The synthetic protocol is summarized in Scheme 1. Reaction of calix[4]arene (**1**) with 3 equivalent of ethyl bromoacetate in the presence of excess amount of CaH_2 or BaO as base and template yielded only the hydrolytic decomposition product instead of triester (**2**) reported in the literature.^{6,7} Conversion of triacid to triester derivative was accomplished by refluxing in EtOH with catalytic amount of *p*-toluenesulfonic acid. Nitration was conducted with the treatment of 70% HNO_3 in the presence of HOAc at 0°C. Under these conditions, only one nitro group was introduced at the *para* position of the phenol ring of calix[4]arene without ester group. Further esterification of the remaining phenol group of **3** kept the tetraester in conic conformation. Hydrolysis of the mononitro-tetraester derivative, using NaOH or KOH as base, gave a mixture of free acid **4** and Na^+ or K^+ complexes. Only a bulky base, such as tetramethylammonium hydroxide (Me_4NOH), gave a satisfactory NMR spectrum with exclusion of cation complexation. Reduction of **4** using the standard pressurized hydrogenation with Pd-C or Raney-Ni as a catalyst, gave virtually no reduction product; however, in the presence of palladized charcoal, hydrazine hydrate^{8,9} reduced **4** to amine **5** in nearly-quantitative yield within 2 hours. Reaction of the amino compound with thiophosgene¹⁰ gave the title compound, 5-isothiocyanato-25,26,27,28-tetrakis-[(hydroxycarbonyl)methoxy]-calix[4]arene (**6**).

^1H NMR¹¹ of the monofunctionalized calixarene **4** clearly showed cone conformation. Because of its asymmetric structure, four pairs of doublets for the ArCH_2Ar methylene protons (two AX systems) and 3 singlets in the ratio of 2:1:1 for OCH_2COOH groups were found in the spectrum. Amino- and isothiocyanato- derivatives showed simplified pattern for OCH_2COOH groups (2 singlets in the ratio of 3:1). Upon reduction, the singlet at 7.36 ppm for a benzene ring containing a nitro group disappeared, and a new singlet at upper field (6.59 ppm) attributed to the two protons on the aniline ring was found to merge. An upfield shift was also observed for $\text{ArCH}_{\text{ax}}\text{Ar}$ methylene protons, which might be due to small change in conformation.

In summary, monofunctionalization of calix[4]arene-tetracarboxylic acid with an isothiocyanato group can be conducted with an excellent yield according to scheme 1 (total yield up to 22% based on compound **2**). Further work to conjugate this actinium-225 ionophore to monoclonal antibodies and to evaluate the stability, affinity, and specificity of the radiolabeled antibody are currently in progress and will be reported elsewhere. Calixarene-based actinium ionophores will also have other potential biomedical applications such as contrast agents (CA) in magnetic resonance imaging (MRI)^{12–14} and as luminescent labels for bioassay purpose (with chromophoric antenna).^{15–17}

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11. **5-Nitro-26,27,28-tris[(ethoxycarbonyl)methoxy]calix-[4]arene (3):** To a solution of **2** (3.41 g, 5 mmol) in CH_2Cl_2 (100 cm^3) was added a mixture of 70% HNO_3 (5 cm^3 , 52 mmol) and glacial acetic acid (7 cm^3). The color of the reaction mixture immediately turned to dark purple. Within 30 min. the color of the mixture changed to yellowish at which point water (50 cm^3) was added and the organic layer was evaporated and the residue recrystallized from CHCl_3 - $\text{C}_2\text{H}_5\text{OH}$ (1/5, v/v) to give **3**. (2.04 g, 56%). Mp: 157–159°C. ^1H NMR (CDCl_3): 1.1–1.4 (m, 9H, CH_3), 3.32 (d, $J = 13.4$ Hz, 2H, ArCH_2Ar eq), 3.41 (d, $J = 13.8$ Hz, ArCH_2Ar eq), 4.1–4.6 (m, 6H, OCH_2CH_3), 4.72 (s, 2H, ArOCH_2), 4.77 (d, $J = 13.8$ Hz, ArCH_2Ar , ax), 4.91 (d, $J = 13.4$ Hz, ArCH_2Ar , ax), 4.95 (s, 4H, ArOCH_2), 6.5–7.0 (m, 9H, ArH), 7.94 (s, 2H, ArH meta), 8.03 (s, 1H ArOH). m/z (FAB): 727 (100%, M^+). Anal. Calcd for $\text{C}_{40}\text{H}_{41}\text{NO}_{12}$: C, 66.02; H, 5.64; N, 1.93. Found: C, 66.15; H, 5.48; N, 1.87%. **5-Nitro-25,26,27,28-tetrakis[hydroxycarbonyl]methoxy]-calix[4]arene (4):** To a solution of **3** (1.45 g, 2 mmol) in THF-DMF (5:1 v/v, 50 cm^3) was added NaH (0.2 g, 8 mmol) and the mixture was heated under reflux for 30 min, after which $\text{BrCH}_2\text{COOEt}$ (0.84 g, 5 mmol) was added and the reaction mixture was refluxed for further 5 h. After cooling, the mixture was cautiously poured over ice-cold HCl (6 M, 30 cm^3). The solvent was evaporated and the residue was partitioned between CH_2Cl_2 (100 cm^3) and HCl (0.2 M, 100 cm^3). The organic layer was evaporated and the residue was crystallized from 90% EtOH to afford the tetraester as yellow crystals. (1.09 g, 67%). To a solution of tetraester (1 g, 1.23 mmol) in THF (20 cm^3) was added Me_4NOH (25% solution in methanol 6 cm^3 , 14 mmol) and water (20 cm^3). The mixture was refluxed for 12 h and was concentrated to dryness under reduced pressure. The residue was treated with 10% HCl (10 cm^3) to form a precipitate which was filtered and recrystallized from 50% methanol to afford **4** (0.70 g, 81 %). Mp: 297–298°C. ^1H NMR (CD_3OD) 3.26 (2H, d, $J = 13.8$ Hz, ArCH_2Ar eq), 3.38 (2H, d, $J = 13.8$ Hz, ArCH_2Ar eq), 4.59 (2H, s, ArOCH_2), 4.76 (2H, s, ArOCH_2), 4.80 (4H, s, ArOCH_2), 4.91 (2H, d, $J = 13.8$ Hz, ArCH_2Ar ax), 5.04 (2H, d, $J = 13.8$ Hz, ArCH_2Ar ax), 6.35 (1H, t, $J = 7.5$ Hz, ArH), 6.48 (2H, d, $J = 7.5$ Hz, ArH), 6.88 (2H, t, $J = 7.7$ Hz, ArH), 7.03 (4H, d, $J = 7.7$ Hz, ArH), 7.36 (s, 2H, ArH). HPLC: (C_{18} reversed phase column, binary linear gradient of 0–100% B/35 min; solvent A = 0.05 mol dm^{-3} $\text{Et}_3\text{N}/\text{HOAc}$, pH = 5.5; solvent B = MeOH) 21.7 min. m/z (FAB): 724 (100%, $\text{M}+\text{Na}$). Anal. Calcd for $\text{C}_{36}\text{H}_{31}\text{NO}_{14}$: C, 61.63; H, 4.42; N, 2.00. Found: C, 61.78; H, 4.25; N, 2.11%. **5-amino-25,26,27,28-tetrakis-[(hydroxycarbonyl)methoxy]calix[4]arene (5):** To a solution of **4** (0.5 g, 0.71 mmol) in EtOH (50 cm^3) was added 10% palladium on activated carbon (50 mg) under argon atmosphere, the resulting mixture was refluxed for 2 hours after which the solution was almost colorless. Pd-C catalyst filtered and washed with EtOH, the combined filtrate was evaporated to give almost pure **5** (0.44 g, 92%). Mp: 323°C (decomp.). ^1H NMR (D_2O) 3.31 (2H, d, $J = 12.6$ Hz, ArCH_2Ar eq), 3.47 (2H, d, $J = 12.6$ Hz, ArCH_2Ar eq), 4.33 (s, 2H, ArOCH_2), 4.40 (s, 6H, ArOCH_2), 4.50 (2H, d, $J = 13.8$ Hz, ArCH_2Ar ax), 5.64 (2H, d, $J = 13.8$ Hz, ArCH_2Ar ax), 6.59 (s, 2H, ArH), 6.8 (m, 3H, ArH), 7.17 (d, br, 6H, ArH). HPLC: 19.1 min. m/z (FAB): 694 (100%, $\text{M}+\text{Na}$). Anal. Calcd for $\text{C}_{36}\text{H}_{33}\text{NO}_{12}$: C, 64.38; H, 4.92; N, 2.09. Found: C, 64.59; H, 4.84; N, 2.11%. **5-isothiocyano-25,26,27,28-tetrakis[(hydroxycarbonyl)methoxy]calix[4]arene (6):** To a solution of **5** (0.3 g, 0.45 mmol) in H_2O (10 cm^3) was added thiophosgene (0.5 cm^3 , 2.88 mmol) in CHCl_3 (10 cm^3) and the mixture was stirred overnight at r.t. The organic phase was evaporated and solid filtered to give **6** (0.25 g, 78%). Mp: > 370°C. IR $\nu_{\text{max}}/\text{cm}^{-1}$ 2100 (NCS) and 1660 (C=O). ^1H NMR ($[\text{D}_6]\text{DMSO}$) 3.3–3.6 (4H, m, ArCH_2Ar eq), 4.1–4.4 (4H, m, ArCH_2Ar ax), 4.61 (6H, s, ArOCH_2), 4.82 (2H, s, ArOCH_2), 6.1–7.2 (11H, m, ArH). HPLC: 13.4 min. m/z (FAB): 736 (100%, $\text{M}+\text{Na}$). Anal. Calcd for $\text{C}_{37}\text{H}_{31}\text{NO}_{12}\text{S}$: C, 62.27; H, 4.35; N, 1.96. Found: C, 62.14; H, 4.32; N, 2.05%.

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Scheme 1.

Scheme 2.